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L2: Entry 66 of 69 File: USPT Aug 31, 1993

DOCUMENT-IDENTIFIER: US 5240863 A
TITLE: Method of measuring immunoreactant using electrochemiluminescence

Abstract Paragraph Type 0 (1):
mixing the liquid sample with an excess of a complementary immunoreactant capable of specifically binding to the immunoreactant to allow an immunoreaction to take place, in which the complementary immunoreactant is immobilized on insoluble carrier particles and labeled with an electrochemiluminescent substance that emits an electrochemiluminescent light by electrolytic oxidation in the presence of activated oxygen,

Brief Summary Paragraph Right (4):
Heretofore, it is known for measurement of an immunoreactant such as antigen or antibody in a liquid sample, to allow the immnoreactant to react with a complementary immnoreactant (antibody or antigen) which has been previously labeled with an electrochemiluminescent substance such as luminol or pyrene. The antigen-antibody reaction occurring between the two immnoreactants suppresses the electrochemiluminescence of the electrochemiluminescent substance labeled on the reacted complementary immunoreactant. Thus, it is possible to measure the concentration of the immunoreactant in the liquid sample by measuring reduction in emission of the electrochemiluminescent light (Y. Ikariyama et al., Biochem. Biophys. Res. Commun., 128, 987 (1985)).

Brief Summary Paragraph Right (8):
mixing the liquid sample with an excess of a complementary immnoreactant capable of specifically binding to said immunoreactant to allow an immunoreaction to take place, said complementary immunoreactant having been immobilized on insoluble carrier particles and labeled with an electrochemiluminescent substance that emits an electrochemiluminescent light by electrolytic oxidation in the presence of an activated oxygen,

Detailed Description Paragraph Right (1):
As to insoluble carrier particles used in the present invention, there is no particular restriction if the particles are solid particles that are stable and insoluble in an aqueous medium, and any carrier particles known as carrier for antigens or antibodies conventionally used in antigen-antibody reactions can be used without any particular restriction. Preferable carrier particles include, for example, organic polymer finely divided particles such as organic polymer latexes obtained by emulsion polymerization, e.g., finely divided particles of a polystyrene, a styrene/butadiene copolymer, a styrene/methacrylic acid copolymer, a polyglycidyl methacrylate, and an acrolein/ethylene glycol dimethacrylate copolymer; inorganic oxide finely divided particles such as finely divided particles of silica, silica-alumina, and alumina; Fe.sub.3 O.sub.4, .gamma.-Fe.sub.3 O.sub.4, Co-.gamma.-Fe.sub.2 O.sub.3, (NiCuZn)O-Fe.sub.2 O.sub.3, (CuZn)O-Fe.sub.2 O.sub.3, (Mn-Zn)O-Fe.sub.2 O.sub.3, (NiZn)O-Fe.sub.2 O.sub.3, SrO-6Fe.sub.2 O.sub.3, BaO-6Fe.sub.2 O.sub.3, Fe.sub.3 O.sub.4 coated with SiO.sub.2 (having a particle diameter of about 150-500 .ANG.) [see "Enzyme Microb. Technol.," vol. 2, pp 2-10 (1980)], composite finely divided particles of various polymer materials, e.g., nylons, polycrylamides, and proteins, with ferrite, and magnetic metal finely divided particles. Generally, the particle diameter of these insoluble carrier particles is preferably 1.0 .mu.m or below. Among the above-mentioned particles, the magnetic finely divided particles diameter preferably has a particle diameter of 50 to 500

.ANG. where they are used singly. The other finely divided particles including composite particles of the magnetic finely devided particles with a polymer material preferably have a particle diameter of about 0.05 to 0.4 .mu.m.

Detailed Description Paragraph Right (3):
The complementary immunoreactant immobilized on the insoluble carrier particles that can be used in the present invention can be produced by immobilizing a prescribed complementary immunoreactant to the above insoluble carrier particles. A variety of immobilizing techniques are known, and any of the methods using physical adsorption and the methods using formation of chemical covalent boding can be used. Generally, physical adsorption is suitable to immobilize a protein highly capable of being physically adsorbed such as antibodies or a protein having a high molecular weight, while formation of chemical covalent bonding is suitable to immobilize hormones and haptens which are low in capability of being physically adsorbed. The immobilizing method may be selected suitably depending, for example, on the properties of the complementary immunoreactant to be immobilized. Where inorganic oxide particles or magnetic finely divided particles out of the above-mentioned finely divided particles are used as the insoluble carrier particles, it is recommended to introduce functional groups onto the insoluble carrier particles as conventionally performed, for example, by treating with a silane coupling agent or coating with protein A produced by staphylococcus aureus.

Detailed Description Paragraph Right (4):
Generally, as a dispersing medium a buffer is used, and insoluble carrier particles and a complementary immunoreactant is mixed therein, if desired, after a dispersing agent is added. Although there is no particular restriction on the dispersing agent used therein, in view of the stability of the insoluble carrier particles during their storage and the reproducibility of the reaction at the time of agglutination, a buffer such as a glycine/sodium hydroxide buffer, a Tris-HCl buffer, an ammonium chloride/ammonia buffer, and a phosphate buffer can be suitably used.

Detailed Description Paragraph Right (5):
The antibody or antigen used as the complementary immunoreactant to be immobilized to the insoluble carrier particles needs to have complementary relationship with a particular immunoreactant, i.e., antigen or antibody to be measured. Examples of such an antigen or antibody to be measured or used as the measuring means are shown below, but the present invention is not restricted to them at all.

Detailed Description Paragraph Right (8):
As the electrochemiluminescent substance to be used as a marker, any of those known to exhibit electrochemiluminescence by electrolytic oxidation in the presence of an activated oxygen, can be used. Examples include luminol, pyrene, luciferin and their electrochemiluminescent derivatives such as isoluminol, N-aminohexyl-N-ethylluminol, N-aminobutyl-N-ethyl isoluminol (ABEI) and activated ester derivatives of ABEI. Among these, preferred is luminol. The electrochemiluminescent substance as a marker may be affixed to the insoluble carrier particles or on the immobilized complementary immunoreactant or on both of them.

Detailed Description Paragraph Right (13):
The method of the present invention is suitable in the cases where the concentration of the immunoreactant to be detected is in the range of 10.sup.-11 to 10.sup.-6 g/ml. The immunoreactant immobilized on an insoluble career particles are normally mixed with the liquid sample so that the concentration thereof may be in the range of 0.01 to 1 mg/l, preferable about 0.1 mg/l, and at that time the complementary immunoreactant on the carrier particles is normally present in an amount of 10 to 1,000 .mu.g, preferably 100 to 400 .mu.g per gram of the carrier particles.

Detailed Description Paragraph Right (14):
If the liquid sample contains an immunoreactant to be detected, the labeled and immobilized complementary immunoreactant reacts with the immunoreactant. The quantity of the reacted labeled and immobilized complementary immunoreactant depends on the quantity of the immunoreactant present in the liquid sample. The reacted electrochemiluminescently labeled and immobilized complementary immunoreactant is difficult to participate in electrochemiluminescence which will be caused by subsequent application of an electric voltage. The suppression of the

electrochemiluminescence is far more intense as compared with the case where the complementary immunoreactant is not immobilized on carrier particles. Therefore, a slight change in the quantity of the labeled and immobilized complementary immunoreactant that has undergone the antigen-antibody reaction results in a great change in emission quantity of electrochemifluorescent light. That is, the change rate of emission of electrochemiluminescent light depending on the change in concentration of an immunoreactant to be detected in a liquid sample, is large.

Detailed Description Paragraph Right (16):
The labeled and immobilized complementary immunoreactant is generally added in the form of an aqueous suspension to a liquid sample. There is no particular restriction on the method of the addition. A given amount of the suspension containing the insoluble carrier particles in a given concentration may be added to a given amount of a liquid sample, or alternatively the latter may be added to the former.

Detailed Description Paragraph Right (17):
If magnetic finely divided particles are used as the insoluble carrier particles, for example, it is possible that the suspension disclosed in Japanese Preexamination Patent Application (kokai) No. 63-90766(1988), that is, a suspension wherein magnetic finely divided particles having a particle diameter of 50 to 500 .ANG. to which a complementary immunoreactant has been immobilized are dispersed in an isotonic aqueous salt solution containing 0.1 wt. % or more of a surface active agent with the amount of the isotonic aqueous solution being 5 ml or more per mg of the magnetic finely divided particles, is previously prepared and is added to a liquid sample. Such a suspension is convenient because it is highly stable and good in storage stability. As the isotonic aqueous salt solution, for example, a 0.9% NaCl solution or a 0.025M aqueous sucrose solution can be used. The surface active agent to be added to the isotonic aqueous salt solution includes surface active agents having a group --COOH or --COO.sup.-, such as Tween 80. It is required that the concentration of the surface active agent in the isotonic aqueous salt solution is 0.1 wt. % or over, preferably 0.1 to 1.0 wt. %.

Detailed Description Paragraph Right (18):
In the method of the present invention, to disperse the insoluble carrier particles into a liquid sample, for example, ultrasonic waves can be used. The dispersion treatment of the sample liquid into the suspension allows the immunoreactant present in the liquid sample to bind to the complementary immunoreactant immobilized on the carrier particles to thereby produce a complex of the immunoreactant/complementary immunoreactant/carrier particles. For this treatment, various methods can be used, for example, a method of forming a complex of immunoreactant/complementary immunoreactant/magnetic finely divided particles by applying an alternating field as described in Japanese Pre-examination Patent Application (kokai) No. 02-281142(1990) and methods using stirring can be used, and there is no particular restriction. Presumably, once said complex is thus formed, when an electric voltage is applied the electrochemiluminescent substance incorporated in the complex is difficult to participate in the electrochemiluminescence due to the steric hindrance by the insoluble carrier particles in comparison with the case where the complementary immunoreactant is not immobilized to carrier particles.

Detailed Description Paragraph Right (21):
(1) After 2 mg of finely divided magnetic particles having a particle diameter of 120 .ANG. was treated with -aminopropylethoxysilane and then with glutaraldehyde, 2 mg of anti-human IgG antibody was added thereto, thereby immobilizing the antibody to the magnetic finely divided particles through covalent bonding. Then the anti-human IgG-immobilized magnetic finely divided particles were reacted with 1 mg of diazotized luminol to label the anti-human IgG-immobilized magnetic finely divided particles with luminol.

Detailed Description Paragraph Right (22):
(2) The anti-human IgG-immobilized magnetic finely divided particles labeled with luminol was dispersed in a PBS (phosphate buffered saline) containing 0.6 wt. % of Tween 80 and having a pH of 7.4 so that the concentration of the magnetic finely divided particles might be 0.2 mg/ml. The thus obtained dispersion was mixed with each of a control solution containing no human IgG (0 ng/ml) and 6 sample solutions whose human IgG concentrations were known, i.e., 1.times.10.sup.-2 ng/ml, 1.times.10.sup.-1

ng/ml, 1.times.10.sup.0 ng/ml, 1.times.10.sup.1 ng/ml, 1.times.10.sup.2 ng/ml and 1.times.10.sup.3 ng/ml, respectively, with the volume ratio being 1:1, and the antigen-antibody reaction was thereby allowed to proceed. The reaction was effected at room temperature for 10 min with an alternating magnetic field having a magnetic flux density of 200 gauss and a pulse width of 0.5 sec being applied.

Detailed Description Paragraph Right (23):
(3) The cell for measurement of electrochemiluminescence schematically shown in FIG. 1 was used. In the cell, two plates constituting the wall 1 and the wall 2 are disposed 1 mm apart opposite to each other so as to form a inner space 3 with a volume of 500 .mu.m, and the both sides and the bottom 4 are sealed. On the inner surface of the wall 2, a Pt-working electrode 5 (thickness: 0.3 mm; area: 0.32 cm.sup.2) and a Pt-counter electrode 6 (thickness: 0.3 mm; area: 0.6 cm.sup.2) and a reference electrode 6 (Ag/AgCl electrode) are provided. In the area in the wall 1 opposite to the working electrode 5, a transparent window 8 made of polymethacrylate with a thickness of 1 mm is provided. This window has a light transmittance of more than 90% at 425 nm. For the measurement of the quantity of emission of luminescence, the reaction mixture and a PBS containing hydrogen peroxide in an amount of 10.sup.-3 mol per liter were mixed with the volume ratio being 1:1, then after the mixed liquid was allowed to stand for 10 min at room temperature, 500 .mu.l of the liquid was placed in the above cell, an electric voltage was applied to the working electrode for 15 sec using an electric potential generating apparatus so that the electric potential of the working electrode might be +1200 mV with respect to the electrical potential of the Ag/AgCl reference electrode, thereby causing the luminol to emit luminescent light. The emission quantity of luminescence was measured by a photon counting system which uses as an optical detector a photomultiplier disposed outside and close to the window 8.

Detailed Description Paragraph Right (25):
A PBS containing 50 .mu.m/ml of a luminol-labeled anti-human IgG antibody which had been labeled by diazotizing luminol was prepared. 500 .mu.l of this labeled antibody solution was mixed with 500 .mu.l of each of 5 sample solutions having human IgG concentrations of 1.times.10.sup.-2 mg/ml, 1.times.10.sup.-1 mg/ml, 1.times.10.sup.0 mg/ml, 1.times.10.sup.1 mg/ml and 1.times.10.sup.2 mg/ml, respectively, and each of the mixtures was stirred slowly at room temperature for 3 hours to allow antigen-antibody reaction to take place. Each of the reaction mixtures was mixed with a PBS containing 10.sup.-3 mol of hydrogen peroxide per liter with the volume ratio being 1:1. After the mixture was then allowed to stand for 10 min at room temperature, the measurement of emission of luminescent light was carried out in the same way as in Example 1.

Detailed Description Paragraph Right (27):
Polystyrene latex particles having an average particle diameter of 0.023 .mu.m were diluted with a Tris-HCl buffer (pH: 7.5) to prepare a suspension having a latex concentration of 1 wt. %. Then, anti-human IgG was diluted with Tris-HCl buffer (pH: 7.5) to prepare a solution having a protein concentration of 2 mg/ml. 1 ml of the anti-human IgG was added to 1 ml of the above latex suspension and they were allowed to react for at 37.degree. C. for 2 hours. Similarly, 1 ml of a luminol solution (having a concentration of 0.1 mmol/1) was added to that solution and the reaction was effected at 37.degree. C. for 2 hours. Further, bovine serum albumin was added so that the final concentration might be 0.05%, then centrifuging was effected, the supernatant was removed, and the sediment was redispersed in Tris-HCl buffer (pH: 7.5) to prepare an anti-human IgG-immobilized latex labeled with luminol which had a latex concentration of 0.08 wt. %.

Detailed Description Paragraph Center (2):
Insoluble Carrier Particles

CLAIMS:

1. A method of detecting the presence or amount of an immunoreactant in a liquid sample, comprising the steps of:

mixing the liquid sample with an excess of a complementary immunoreactant capable of specifically binding to said immunoreactant to allow an immunoreaction to take place,

said complementary immunoreactant having been immobilized on insoluble carrier particles and labeled with an electrochemiluminescent substance that emits an electrochemiluminescent light by electrolytic oxidation in the presence of activated oxygen;

applying an electric voltage to a pair of electrodes between which the mixture obtained above is placed, in the presence of activated oxygen to allow electrochemiluminescence to take place;

measuring the emission of the electrochemiluminescent light; and

correlating the presence or amount of said immunoreactant with an amount of measured electrochemiluminescent light.

5. The method according to claim 1, wherein said insoluble carrier particles are organic polymer finely divided particles, inorganic oxide finely divided particles, composite finely divided particles of a polymer material and ferrite, or magnetic metal finely divided particles.

6. The method according to claim 1, wherein said insoluble carrier particles have a particle diameter of 1.0 .mu.m or less.

7. The method according to claim 1, wherein said electrochemiluminescent substance is luminol, pyrene, luciferin, or an electrochemiluminescent derivative thereof.

L2: Entry 63 of 69 File: USPT Jun 14, 1994

DOCUMENT-IDENTIFIER: US 5320944 A
TITLE: Immunoassay using magnetic particle

Abstract Paragraph Left (1):
An immunoassay method using magnetic particles comprising a core and a coating layer on the surface thereof, The core comprises an organic polymer and the coating layer comprises an iron oxide type ferrite coating layer. An antigen or an antibody is bound onto the surface of the coating layer and the particle has a particle size of 0.2 to 3 .mu.m.

Brief Summary Paragraph Right (1):
This invention relates to an immunoassay using magnetic particles, more specifically to an enzyme immunoassay using magnetic particles in which a core comprises an organic polymer and a surface comprises a ferrite coating layer composed of iron oxide and an antigen or antibody is bound thereto, and a particle size of which is 0.2 to 3 .mu.m.

Brief Summary Paragraph Right (4):
The present inventors have studied intensively to overcome these problems and as the results, they have found that when magnetic particles which comprise a core composed of an organic polymer and a surface composed of an iron oxide type ferrite coating layer, with a particle size of 0.2 to 3 .mu.m and an antigen or an antibody is bound on the particles are employed for an immunoassay method, measurement results excellent in reproducibility could be obtained. This can be also understood from the fact that said magnetic particles are stable for a long period of time so that preservation for a long period of time can be done.

Detailed Description Paragraph Right (2):
The magnetic particles to be used in the present invention can be prepared by using an organic polymer as a core and subjecting to an iron oxide type ferrite coating, and then binding an a or an antibody to the resulting magnetic particles. The organic polymer comprises at least one polymer a polystyrene and or at least one of an acrylate and a methacrylate (hereinafter (meth)acrylates).

Detailed Description Paragraph Right (14):
The present invention relates to magnetic particles obtained by binding an antigen or an antibody to the ferrite coated particles or further polymer compoundtreated particles obtained by the above method. As the antibody to be used, there may be mentioned, for example, a chemical such as theophylline, phenytoin and a low molecular hormone such as thyroxine, estrogen and estradiol; a cancer marker such as CEA and AFP; a virus antigen such as HIV, ATLA and HBV; a high molecular hormone such as TSH and insulin; a cytocain such as IL-1, IL-2 and IL-6; various kinds of gloss factor such as EGF and PDGF; and further an antibody to a suitable DNA, RNA, etc. of the above viruses. Also, as the antigen to be used, there may be mentioned a virus such as HIV, ATLA and HBV; DNA of the above viruses; a high molecular hormone such as insulin and TSH.

Detailed Description Paragraph Right (19):
The enzyme immunoassay method according to the present invention is, for example, to carry out by reacting an antibody-bound magnetic particle and an enzyme-labelled antibody for 10 minutes to 3 hours. A reaction temperature when practicing the reaction is 4.degree. C. to 40.degree. C., and preferably 25.degree. C. to 38.degree. C. After washing an unreacted enzyme-labeled antibody, an amount of a ligand of

specimen can be determined by measuring an amount of an antigenbound enzyme bound to a solid phase, by adding an enzyme substrate and measuring an activity thereof. An enzyme to be used in the of the invention may include peroxidase, alkaline phosphatase, .beta.-galactosidase and glucoseoxidase. At this time, it is needless to say that a substrate to be used should be that which is suitable for an enzyme to be used. As such substrates, there may be used, for example, ABTS, luminol-H202 (for peroxidase), 3-(2'-pyro-tricyclo[3.3.1.1.sup.3,7]decan)-4-methoxy-4(3"-phosphoryloxy) phenyl-1,2-dioxetane disodium salt, p-nitrophenylphosphate and methylumbelliferyl phosphate (for alkaline phosphatase), p-nitrophenyl-.beta.-o-glactose and methylumbelliferyl-.beta.-o-galactose (for .beta.-galactosidase). The measurement can be carried out by reacting at room temperature to 40.degree. C. for 1 minute to 18 hours, and then measuring an amount of color, fluorescence or luminescence generated. As the other method, the so-called rate method in which it is carried out at a temperature range of 4.degree. C. to 40.degree. C. under heating may be employed.

Detailed Description Paragraph Right (21):
Also, radiolabelling of an antigen or an antibody can be readily prepared by the already available Bolton-Hunter reagent. It can be prepared by, for example, adding the Bolton-Hunter reagent to an antigen or an antibody solution dissolved in a 0.1 M sodium hydrogen carbonate aqueous solution, and after 1 to 2 hours, removing unreacted Bolton-Hunter reagent by using a desalting column of G-25, etc. In addition, radiolabelling of 1251 can be easily carried out by employing the chloramine T method or the iododine method. For effecting the immuno reaction, a sample is added to the magnetic particles of the present invention, and reacted at 4.degree. C. to 40.degree. C., preferably 20.degree. C. to 38.degree. C. for 1 to 18 hours. Thereafter, washing is carried out by a physiological salt solution or distilled water, radiolabelled antibody is added to magnetic particles and reacted at 4.degree. C. to 40.degree. C., preferably 20.degree. C. to 38.degree. C. for 1 to 18 hours, washed with a physiological salt solution or distilled water and then countering its radioactivity. A scintillation counter can be used for the measurement.

Detailed Description Paragraph Right (23):
Similarly, measurement of the antibody can be carried out by using the magnetic particles of the present invention, mixing these particles with a sample to react them at a room temperature to 37.degree. C. for one minute to 18 hours, washing with a physiological salt solution or distilled water, and then adding labelled-anti-human immunoglobulin antibody to react at a room temperature to 37.degree. C. for 1 minute to 18 hours, washing and measuring the activity of the labelled substance.

Detailed Description Paragraph Right (24):
The present invention is an enzyme immunoassay method using particles comprising magnetic particles composed of an organic polymer as a core and a ferrite layer deposited on the surface thereof, and an antigen or antibody bound on the surface of the ferrite layer. These particles may be used as a solid phase of an immunoassay method.

Detailed Description Paragraph Right (34):
To a sample containing 15 .mu.l of TSH (0, 10 .mu.U/ml) was mixed 20 .mu.l of alkali phosphatase conjugate (conjugate concentration: 0.5 .mu.g/ml, 0.1 M Tris-hydrochloric acid, 2% BSA, 1 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2, pH: 7.5) to which anti-TSH Fab' is bound, and then 500 .mu.l (0.02% solution) of ferrite particles prepared in Example 2 on which anti-TSH mouse IgG was coated was mixed to the above mixture, and the resulting mixture was allowed to stand at room temperature for 20 minutes. A tube containing the above mixture was contacted with a magnet having a surface magnetic field of 3000 gauss to attract ferrite particles and the supernatant was removed by decantation. Thereafter, 1 ml of 0.04% physiological salt solution was added to the particles and the mixture was stirred. The tube was again contacted with the above mentioned magnet to separate the particles and a supernatant, and the supernatant was removed by decantation. These operations were repeated three times. To the tube containing these particles was added 200 .mu.l of a substrate solution (0.1 M Tris-hydrochloric acid, 1 .mu.M MgCl2, 0.1 mM ZnCl2, pH: 9.8) containing 100 .mu.g/ml of 3-(2'-pyro-tricyclo[3.3 1.1.sup.3,7]decan)-4-(3"-phosphoryloxy) phenyl-1,2-dioxetane disodium salt (AMPPD) and the mixture was reacted at room temperature. After carrying out the reaction for 17 minutes, the sample was measured by a luminometer (manufactured by Belthold Co.). In Table 1, an S/N ratio of

integrated value for 5 minutes was shown. For comparison, the results wherein ferrite particles produced by Advanced Magnetics Co. [magnetic carrier for Affinity Chromatography (carboxyl group terminated)] were used are also shown.

Detailed Description Paragraph Right (36):
In Table 2, an S/N ratio of integrated value for 5 minutes was shown. For comparison, the results wherein ferrite particles produced by Advanced Magnetics Inc. [magnetic carrier for Affinity Chromatography (carboxyl group terminated)] were used are also shown.

Detailed Description Paragraph Right (38):
In FIG. 1, an S/N ratio of integrated value for 5 minutes was shown. For comparison, the results wherein ferrite particles produced by Advanced Magnetics Inc. [magnetic carrier for Affinity Chromatography (carboxyl group terminated)] were used are also shown.

Detailed Description Paragraph Right (44):
The present invention relates to an enzyme immunoassay method using particles composed of a magnetic particle comprising an organic polymer as a core and a ferrite coatings coated on the surface thereof, and an antigen or antibody bound to the surface of the magnetic particle. The particles according to the present invention have uniform particle size and are excellent in binding state of an antigen or antibody to the particles. Further, the particles according to the present invention have advantages that they are stable for a long period of time and thus can be preserved. An enzyme immunoassay method according to the present invention can be carried out by using the particles rapidly and with high sensitivity.

CLAIMS:

1. An immunoassay method comprising the steps of:

(a) reacting a sample containing an antigen with an antibody which specifically binds to said antigen, so as to bind said antigen to said antibody, wherein said antibody is immobilized on a 0.2 to 3.0 .mu.m magnetic particle comprising a core and a coating layer on the surface of said core, wherein said core comprises an organic polymer and said coating layer comprises a metal oxide formed by adding ferrous ions or ferrous ions and an ion selected from the group consisting of zinc, cobalt, nickel, manganese, copper, vanadium, antimony, lithium, molybdenum, titanium, rubidium, aluminum, silicon, chromium, tin, calcium, cadmium, indium ions and mixtures thereof to deoxidized water in which cores of organic polymer are suspended to form a resulting solution, and subsequently adding an oxidizing agent solution to the resulting solution to provide a ferrite coating on said core;

(b) measuring an amount of bound antigen; and

(c) correlating the amount of bound antigen obtained in step (b) with an amount of antigen in the sample.

2. An immunoassay method comprising the steps of:

(a) reacting a sample containing an antibody with an antigen which specifically binds to said antibody, so as to bind said antibody to said antigen, wherein said antigen is immobilized on a 0.2 to 3.0 .mu.m magnetic particle comprising a core and a coating layer on the surface of said core, wherein said core comprises an organic polymer and said coating layer comprises a metal oxide formed by adding ferrous ions or ferrous ions and an ion selected from the group consisting of zinc, cobalt, nickel, manganese, copper, vanadium, antimony, lithium, molybdenum, titanium, rubidium, aluminum, silicon, chromium, tin, calcium, cadmium, indium ions and mixtures thereof to deoxidized water in which cores of organic polymer are suspended to form a resulting solution, and subsequently adding an oxidizing agent solution to the resulting solution to provide a ferrite coating on said core;

(b) measuring an amount of bound antibody; and

(c) correlating the amount of bound antibody obtained in step (b) with an amount of

antibody in the sample.

11. The immunoassay method according to claim 5, wherein (a) said sample comprises said antigen; (b) said antibody is immobilized on said magnetic particle; and (c) said method further comprises adding an enzyme-labeled antibody, which specifically binds to said antigen to the sample; and separating unreacted enzyme labeled antibody from the sample by washing out said unreacted enzyme-labeled antibody, adding an enzyme substrate specific for the enzyme and measuring the activity of antibody-bound enzyme.

12. The immunoassay method according to claim 6, wherein (a) said sample comprises said antigen; (b) said antibody is immobilized on said magnetic particle; and (c) said method further comprises adding an enzyme-labeled antigen, which specifically binds to said antibody to the sample; and separating unreacted enzyme-labeled antigen from the sample by washing out said unreacted enzyme-labeled antigen, adding an enzyme substrate specific for the enzyme and measuring the activity of antigen-bound enzyme.

13. The immunoassay method according to claim 11, wherein said enzyme substrate is selected from the group consisting of (i) ABTS and luminol-H.sub.2 O.sub.2 for peroxidase; (ii) (2'-pyro-tri-cyclodecab)-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane disodium salt, p-nitrophenylphosphate and methylumbelliferyl phosphate for alkaline phosphatase; and (iii) p-nitrophenyl-.beta.-o-galactose and methylumbelliferyl-.beta.-o-galactose for .beta.-galactosidase.

14. The immunoassay method according to claim 12, wherein said enzyme substrate is selected from the group consisting of (i) ABTS and luminol-H.sub.2 O.sub.2 for peroxidase; (ii) (2'-pyro-tri-cyclodecab)-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane disodium salt, p-nitrophenylphosphate and methylumbelliferyl phosphate for alkaline phosphatase; and (iii) p-nitrophenyl-.beta.-o-galactose and methylumbelliferyl-.beta.-o-galactose for .beta.-galactosidase.

17. The immunoassay method according to claim 3, wherein (a) said sample comprises said antigen; (b) said antibody is immobilized on said magnetic particle; and (c) said method further comprises adding an enzyme-labeled antigen, which specifically binds to said antibody to the sample; and separating unreacted enzyme labeled antigen from the sample by washing out said unreacted enzyme-labeled antigen, adding an enzyme substrate specific for the enzyme and measuring the activity of antigen-bound enzyme.

18. The immunoassay method according to claim 4, wherein (a) said sample comprises said antibody; (b) said antigen is immobilized on said magnetic particle; and (c) said method further comprises adding an enzyme-labeled antibody, which specifically binds to said antibody to the sample; and separating unreacted enzymelabeled antibody from the sample by washing out said unreacted enzyme-labeled antibody, and adding an enzyme substrate specific for the enzyme and measuring the activity of antibody-bound enzyme.

19. The immunoassay method according to claim 17, wherein said enzyme substrate is selected from the group consisting of (i) ABTS and luminol-H.sub.2 O.sub.2 for peroxidase; (ii) (2'-pyro-tricyclo[3.3.1.1..sup.3,7]decan)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane disodium salt, p-nitrophenylphosphate and methylumbelliferyl phosphate for alkaline phosphatase; and (iii) p-nitrophenyl-.beta.-o-galactose and methylumbelliferyl-.beta.-o-galactose for .beta.-galactosidase.

20. The immunoassay method according to claim 18, wherein said enzyme substrate is selected from the group consisting of (i) ABTS and luminol-H.sub.2 O.sub.2 for peroxidase; (ii) (2'-pyro-tricyclo[3.3.1.1.sup.3,7]-decan)-4-methoxy-4-(3"-phosphoryloxy)-phenyl-1,2-dioxetane disodium salt, p-nitrophenylphosphate, and methylumbelliferyl phosphate for alkaline phosphatase; and (iii) p-nitrophenyl-.beta.-o-galactose and methylumbelliferyl-.beta.-o-galactose for .beta.-galactosidase.